

AMENDMENTS TO THE CLAIMS

1. (Original) A method of preparing a DNA molecule, comprising:
 - obtaining at least one DNA molecule;
 - randomly fragmenting the DNA molecule to produce DNA fragments;
 - modifying the ends of the DNA fragments to provide attachable ends;
 - attaching an adaptor having at least one known sequence and a nonblocked 3' end to the ends of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and a 5' end of the adaptor;
 - extending the 3' end of the modified DNA from the nick site; and
 - amplifying a plurality of the adaptor-linked fragments.
2. (Original) The method of claim 1, wherein said at least one DNA molecule is further defined as genomic DNA.
3. (Original) The method of claim 1, wherein said modifying step is further defined as modifying the ends of the DNA fragments to comprise blunt double stranded ends.
4. (Original) The method of claim 1, wherein said modifying step is further defined as modifying the ends of the DNA fragments to comprise an overhang of at least 1 nucleotide.
5. (Original) The method of claim 1, wherein said randomly fragmenting the DNA molecule comprises mechanical fragmentation.
6. (Original) The method of claim 5, wherein said mechanical fragmentation comprises hydrodynamic shearing, sonication, nebulization, or a combination thereof.

7. (Original) The method of claim 1, wherein said randomly fragmenting the DNA molecule comprises chemical fragmentation.

8. (Original) The method of claim 7, wherein said chemical fragmentation comprises acid catalytic hydrolysis, alkaline catalytic hydrolysis, hydrolysis by metal ions, hydroxyl radicals, irradiation, heating, or a combination thereof.

9. (Original) The method of claim 1, wherein said randomly fragmenting the DNA molecule comprises enzymatic fragmentation.

10. (Original) The method of claim 9, wherein said enzymatic fragmentation comprises DNase I digestion.

11. (Original) The method of claim 9, wherein said enzymatic fragmentation comprises Cvi JI restriction enzyme digestion.

12. (Original) The method of claim 8, wherein said chemical fragmentation comprises heating.

13. (Original) The method of claim 1, wherein the modifying step comprises repair of at least one 3' end of the DNA fragment.

14. (Original) The method of claim 13, wherein the modifying step comprises subjecting said DNA fragment to 3' exonuclease activity, 5'-3' polymerase activity, or both.

15. (Original) The method of claim 14, wherein both of said 3' exonuclease activity and said 5'-3' polymerase activity are comprised in the same enzyme.

16. (Original) The method of claim 15, wherein the enzyme comprises Klenow, T4 DNA polymerase, or a mixture thereof.

17. (Original) The method of claim 14, wherein the 3' exonuclease activity comprises Exonuclease III activity and the 3' polymerase activity comprises T4 DNA polymerase activity.

18. (Original) The method of claim 17, wherein following said subjecting step, said DNA fragments are subjected to Klenow, T4 DNA polymerase, or both.

19. (Original) The method of claim 7, wherein said DNA fragments comprise a plurality of ssDNA molecules and said modifying step is further defined as subjecting said ssDNA molecules to a plurality of random primers and DNA polymerase activity, under conditions wherein said blunt double stranded fragments are thereby generated.

20. (Original) The method of claim 19, wherein the random primers further comprise a known sequence at their 5' end.

21. (Original) The method of claim 19, wherein at least one ssDNA molecule comprises a blocked 3' end and wherein said modifying step is further defined as subjecting said ssDNA to 3'-5' exonuclease activity.

22. (Original) The method of claim 19, wherein the random primers are pentamers.

23. (Original) The method of claim 19, wherein the random primers are hexamers.

24. (Original) The method of claim 19, wherein the random primers are septamers.

25. (Original) The method of claim 19, wherein the random primers are octamers.

26. (Original) The method of claim 19, wherein the random primers are nonamers.

27. (Original) The method of claim 19, wherein the random primers are phosphorylated at the 5' end.

28. (Original) The method of claim 19, wherein the random primers are comprised of at least one base analog, at least one backbone analog, or both.

29. (Original) The method of claim 19, wherein said DNA polymerase activity and said 3'-5' exonuclease activity are comprised in the same enzyme.

30. (Original) The method of claim 19, wherein said polymerase is a non strand-displacing polymerase.

31. (Original) The method of claim 19, wherein said polymerase is a strand-displacing polymerase.

32. (Original) The method of claim 30, wherein said non strand-displacing polymerase is T4 DNA polymerase.

33. (Original) The method of claim 31, wherein said strand-displacing enzyme is Klenow or DNA polymerase I.

34. (Original) The method of claim 19, wherein said polymerase comprises nick translation activity.

35. (Original) The method of claim 19, wherein said enzyme is Klenow, T4 DNA polymerase, or DNA polymerase I, or a mixture thereof.

36. (Original) The method of claim 19, wherein said modifying step occurs in the presence of additives known to facilitate polymerization through GC-rich DNA.

37. (Original) The method of claim 36, wherein said additives comprise dimethyl sulfoxide (DMSO), 7-Deaza-dGTP, or a mixture thereof.

38. (Original) The method of claim 1, wherein said modifying step and said attaching step occurs concomitantly.

39. (Original) The method of claim 9, wherein said enzymatic fragmentation occurs in the presence of Mn²⁺ and said modifying step is further defined as subjecting said DNA fragments to 3' exonuclease activity, 5'-3' polymerase activity, or both.

40. (Original) The method of claim 39, wherein both of said 3' exonuclease activity and said 5'-3' polymerase activity are comprised in the same enzyme.

41. (Original) The method of claim 40, wherein said enzyme is Klenow, T4 DNA polymerase, or a mixture thereof.

42. (Original) The method of claim 40, wherein said 3' exonuclease activity is by exonuclease III and said 5'-3' polymerase activity is by T4 DNA polymerase.

43. (Original) The method of claim 42, wherein following said subjecting step, said DNA fragments are subjected to Klenow, T4 DNA polymerase, or both.

44. (Original) The method of claim 9, wherein said enzymatic fragmentation occurs in the presence of Mg²⁺ and said modifying step is further defined as subjecting said DNA fragments to random primers, 5'-3' polymerase activity and 3'-5' exonuclease activity.

45. (Original) The method of claim 44, wherein said 5'-3' polymerase activity and said 3'-5' exonuclease activity are comprised in the same enzyme.

46. (Original) The method of claim 45, wherein said enzyme is Klenow, T4 DNA polymerase, DNA polymerase I, or a mixture thereof.

47. (Original) The method of claim 1, wherein said attaching step is further defined as subjecting said DNA fragments to a blunt end adaptor, a 5' overhang adaptor, a 3' overhang adaptor, or a mixture thereof.

48. (Original) The method of claim 1, wherein said adaptor comprises at least one of the following features:

absence of a 5' phosphate group;

a 5' overhang; or

a blocked 3' base.

49. (Original) The method of claim 48, wherein said 5' overhang comprises about 5 to about 100 bases.

50. (Original) The method of claim 1, wherein said attaching is by ligating the adaptor to the DNA fragment.

51. (Original) The method of claim 50, wherein said ligation is by chemical ligation.

52. (Original) The method of claim 50, wherein said ligation is by enzymatic ligation.

53. (Original) The method of claim 52, wherein said enzymatic ligation is by T4 DNA ligase.

54. (Original) The method of claim 52, wherein said enzymatic ligation is by topoisomerase I.

55. (Original) The method of claim 54, wherein said adaptor is covalently attached to topoisomerase I at a 3' thymidine overhang or a blunt end.

56. (Original) The method of claim 55, wherein said adaptor comprises a sequence of 5'-CCCTT-3'.

57. (Original) The method of claim 54, wherein the DNA fragments are blunt ended and a 3' adenine is added to the blunt ended DNA fragments by polymerase.

58. (Original) The method of claim 1, wherein the adaptor comprises a first primer and a second primer, said first primer greater in length than said second primer.

59. (Original) The method of claim 58, wherein the second primer comprises a blocked 3' end.

60. (Original) The method of claim 1, wherein the adaptor comprises at least one blunt end.

61. (Original) The method of claim 60, wherein the 3' end of at least one primer is blocked.

62. (Original) The method of claim 50, wherein the adaptor comprises one oligonucleotide having two regions complementary to each other, said regions separated by a linker region.

63. (Original) The method of claim 62, wherein when the two complementary regions are hybridized to each other to form a double-stranded region of said adaptor, the end of said double stranded region is a blunt end.

64. (Original) The method of claim 62, wherein said linker region comprises a non-replicable organic chain of about 1 to about 50 atoms in length.

65. (Original) The method of claim 64, wherein said non-replicable organic chain is hexa ethylene glycole (HEG).

66. (Original) The method of claim 1, wherein said extending step comprises subjecting the adaptor-linked fragments comprising the nick to a mixture comprising:

DNA polymerase;

deoxynucleotide triphosphates; and

suitable buffer, under conditions wherein polymerization occurs from the 3' hydroxyl of the nick.

67. (Original) The method of claim 66, wherein the method further comprises heating the mixture.

68. (Original) The method of claim 67, wherein said heating is to a temperature of about 75°C.

69. (Original) The method of claim 66, wherein the polymerase is a strand-displacing polymerase.

70. (Original) The method of claim 66, wherein the DNA polymerase is a thermophilic DNA polymerase.

71. (Original) The method of claim 70, wherein the thermophilic DNA polymerase is Taq polymerase.

72. (Original) The method of claim 66, wherein at least one deoxynucleotide triphosphate is labeled.

73. (Original) The method of claim 1, wherein said amplifying step comprises polymerase chain reaction, said reaction utilizing a primer complementary to a sequence of the adaptor.

74. (Original) The method of claim 73, wherein said primer is labeled.

75. (Original) The method of claim 1, wherein said amplifying step occurs in the presence of additives known to facilitate polymerization through GC-rich DNA.

76. (Original) The method of claim 75, wherein said additives comprise DMSO, 7-Deaza-dGTP, or a mixture thereof.

77. (Original) The method of claim 1, wherein said at least one DNA molecule is comprised in a cell.

78. (Original) The method of claim 1, wherein said at least one DNA molecule is not comprised in a cell.

79. (Original) The method of claim 77, wherein the at least one DNA molecule is cell-free fetal DNA in maternal blood or is cell-free cancer DNA in blood.

80. (Original) The method of claim 1, wherein said obtaining method is further defined as obtaining the at least one DNA molecule from blood, urine, sputum, feces, sweat, nipple aspirate, a fixed tissue sample, immuno-precipitated chromatin, physically isolated chromatin, or a combination thereof.

81. (Original) The method of claim 80, wherein said physically isolated chromatin is isolated by centrifugation, electrophoresis, micro-filtration, affinity capture, or a combination thereof.

82. (Original) The method of claim 2, wherein said genomic DNA comprises bacterial genomic DNA, viral genomic DNA, fungal genomic DNA, plant genomic DNA, or mammalian genomic DNA.

83. (Original) The method of claim 2, wherein said genomic DNA is from an extant species or an extinct species.

84. (Original) The method of claim 1, wherein said at least one DNA molecule comprises a portion of a genome.

85. (Original) The method of claim 1, wherein said adaptor is further defined as a first adaptor having a first known sequence and further comprises a homopolymeric sequence, the method further comprising the following steps:

digesting the amplified adaptor-linked fragments to produce fragmented adaptor-linked fragments;

attaching a second adaptor having a second known sequence to the ends of the fragmented adaptor-linked fragments to produce second adaptor-linked fragments; and

amplifying the second adaptor-linked fragments with a primer complementary to the homopolymeric sequence and a primer complementary to the second known sequence.

86. (Original) The method of claim 85, wherein said homopolymeric sequence is comprised of cytosines.

87. (Original) The method of claim 1, wherein said adaptor is further defined as a first adaptor having a first known sequence, the method further comprising the following steps:

subjecting the amplified adaptor-linked fragments to terminal deoxynucleotidyl transferase to generate a homopolymeric single-stranded tail on said amplified adaptor-linked fragments;

digesting the homopolymeric tailed amplified adaptor-linked fragments;

attaching a second adaptor having a second known sequence to the ends of the digested homopolymeric tailed amplified adaptor-linked fragments that do not comprise the homopolymeric tail, to produce second adaptor-linked fragments; and

amplifying the second adaptor-linked fragments with a primer complementary to the homopolymeric sequence and a primer complementary to the second known sequence.

88. (Original) A method of preparing a DNA molecule, comprising:

obtaining at least one DNA molecule;

attaching a first adaptor having a first known sequence, a homopolymeric sequence and a nonblocked 3' end to the ends of the DNA molecule to produce first adaptor-linked molecules, wherein the 5' end of the DNA molecule is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA molecule and a 5' end of the adaptor;

digesting the adaptor-linked DNA molecules to produce DNA fragments;

attaching a second adaptor having a second known sequence to the ends of the DNA fragments to produce second adaptor-linked fragments; and

amplifying a plurality of the second adaptor-linked fragments.

89. (Original) A method of preparing a DNA molecule, comprising:

obtaining a plurality of DNA molecules, said DNA molecules defined as fragments from at least one larger DNA molecule;

modifying the ends of the DNA fragments to provide attachable ends;

attaching an adaptor having a known sequence and a nonblocked 3' end to both ends of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and a 5' end of the adaptor;

extending the 3' end of the modified DNA from the nick site; and

amplifying a plurality of the adaptor-linked fragments.

90. (Original) The method of claim 89, wherein said at least one larger DNA molecule comprises genomic DNA.

91. (Original) A method of amplifying a genome, comprising the steps of:

obtaining at least one DNA molecule;

randomly fragmenting the DNA molecule to produce DNA fragments;

modifying the ends of the DNA fragments to provide attachable ends;

attaching an adaptor having a known sequence and a nonblocked 3' end to the ends of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and 5' end of the adaptor;

extending the 3' end of the modified DNA from the nick site; and

amplifying a plurality of the adaptor-linked fragments.

92. (Original) A method of generating a library, comprising the steps of:

obtaining at least one DNA molecule;

randomly fragmenting the DNA molecule to produce DNA fragments;

modifying the ends of the DNA fragments to provide attachable ends;

attaching an adaptor having a known sequence and a nonblocked 3' end to both ends of a plurality of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the modified DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site between the juxtaposed 3' end of the DNA and 5' end of the adaptor;

extending the 3' end of the modified DNA from the nick site.

93. (Original) The method of claim 92, wherein said method further comprises amplifying a plurality of the adaptor-linked fragments.

94. (Original) A method of preparing at least one DNA molecule, comprising:

admixing together:

an endonuclease;

a ligase;

an adaptor; and

a buffer, under conditions wherein said DNA molecule is cleaved by said endonuclease to generate a plurality of DNA fragments, a plurality of the ends of which are ligated to said adaptor.

95. (Original) The method of claim 94, wherein the method consists essentially of one step.

96. (Original) The method of claim 94, wherein the cleavage and ligation occur substantially concomitantly.

97. (Original) The method of claim 94, further defined as the ligation occurring under the same reaction conditions as the cleavage.

98. (Original) The method of claim 94, wherein the ligation step occurs without changing the buffer following the cleavage step.

99. (Original) The method of claim 94, wherein the method lacks DNA precipitation.

100. (Original) The method of claim 94, wherein said DNA molecule is further defined as a genome.

101. (Original) The method of claim 94, wherein said endonuclease is deoxyribonuclease I or a Cvi restriction endonuclease.

102. (Original) The method of claim 94, wherein said ligase is T4 DNA ligase.

103. (Original) The method of claim 94, wherein said adaptor is a blunt end adaptor, a 5' overhang adaptor, a 3' overhang adaptor, or a mixture thereof.

104. (Original) The method of claim 94, wherein the adaptor comprises a first primer and a second primer, said first primer greater in length than said second primer.

105. (Original) The method of claim 104, wherein said first primer lacks a 5' phosphate, said second primer lacks a 5' phosphate group, or both first and second primers lack 5' phosphate groups.

106. (Original) The method of claim 94, wherein the buffer comprises a divalent cation, a salt, adenosine triphosphate, dithiothreitol, or a mixture thereof.

107. (Original) The method of claim 94, wherein the conditions comprise a large molar excess of linkers to DNA fragment ends.

108. (Original) The method of claim 107, wherein the large molar excess is at least about 10-fold to about 100-fold.

109. (Original) The method of claim 94, wherein said method further comprises amplifying the DNA fragments using a primer complementary to the adaptor.

110. (Original) A method of generating a library of DNA molecules comprising:

admixing together:

at least one DNA molecule;

an endonuclease;

a ligase;

an adaptor; and

a buffer, under conditions wherein said DNA molecule is cleaved by said endonuclease to generate a plurality of DNA fragments, a plurality of the ends of which are ligated to said adaptor.

111. (Original) The method of claim 110, wherein said method consists essentially of one step.

112.-118. (Cancel)

119. (Original) A method of amplifying at least one RNA molecule, comprising the steps of:

obtaining at least one RNA molecule;

reverse transcribing said RNA molecule to produce a cDNA molecule;

randomly fragmenting the cDNA molecule to produce DNA fragments;

modifying the ends of the DNA fragments to provide attachable ends;

attaching an adaptor having a known sequence and a nonblocked 3' end to the ends of the modified DNA fragments to produce adaptor-linked fragments, wherein the 5' end of the DNA is attached to the nonblocked 3' end of the adaptor, leaving a nick site at the juxtaposed 3' end of the DNA and a 5' end of the adaptor;

extending the 3' end of the modified DNA from the nick site; and

amplifying a plurality of the adaptor-linked fragments.

120. (Original) A method of amplifying a population of DNA molecules comprised in a plurality of populations of DNA molecules, said method comprising the steps of:

obtaining a plurality of populations of DNA molecules, wherein at least one population in said plurality comprises DNA molecules having in a 5' to 3' orientation the following:

a known identification sequence specific for said population; and

a known primer amplification sequence; and

amplifying said population of DNA molecules by polymerase chain reaction, said reaction utilizing a primer for said identification sequence.

121. (Original) The method of claim 120, wherein said obtaining step is further defined as:

obtaining a population of DNA molecules, said molecules comprising a known primer amplification sequence;

amplifying said DNA molecules with a primer having in a 5' to 3' orientation the following:

the known identification sequence; and

the known primer amplification sequence; and

mixing said population with at least one other population of DNA molecules.

122. (Original) The method of claim 120, wherein said population of DNA molecules is a genome.

123. (Original) A method of amplifying a population of DNA molecules comprised in a plurality of populations of DNA molecules, said method comprising the steps of:

obtaining a plurality of populations of DNA molecules, wherein at least one population in said plurality comprises DNA molecules, wherein the 5' ends of said DNA molecules comprise in a 5' to 3' orientation the following:

a single-stranded region comprising a known identification sequence specific for said population; and

a known primer amplification sequence; and

isolating said population through binding of at least part of the single stranded known identification sequence of a plurality of said DNA molecules to a surface; and

amplifying the isolated DNA molecules by polymerase chain reaction, said reaction utilizing a primer for said primer amplification sequence.

124. (Original) The method of claim 123, wherein said obtaining step is further defined as:

obtaining a population of DNA molecules, said molecules comprising a known primer amplification sequence;

amplifying said DNA molecules with a primer comprising in a 5' to 3' orientation the following:

the known identification sequence;

a non-replicable linker; and

the known primer amplification sequence; and

mixing said population with at least one other population of DNA molecules.

125. (Original) The method of claim 123, wherein said isolating step is further defined as binding at least part of the single stranded known identification sequence to an immobilized oligonucleotide comprising a region complementary to the known identification sequence.

126.-139. (Cancel)

140. (Original) A method of preparing a DNA molecule, comprising:

obtaining a population of DNA molecules having ligatable ends of unknown nature;

providing to said population one or more known forms of adaptors, wherein said adaptors each comprise at least one known sequence and at least one oligonucleotide having a 3' extendable end;

determining ligatability of said one or more known forms of adaptors to said DNA molecules; and

ligating said known one or more forms of adaptors to said DNA molecule.

141. (Original) The method of claim 140, wherein said determining step is further defined as identifying a ratio of ligatable forms of adaptors corresponding to the nature of the ends of the DNA molecules in the population, and wherein said ligating step is further defined as introducing to said population a plurality of said adaptors in said ratio.

142. (Original) The method of claim 140, wherein said ligatability of said one or more forms of adaptors are determined separately.

143. (Original) The method of claim 140, wherein said method further comprises the step of extending the 3' end of said oligonucleotide by polymerization to produce an extended product.

144. (Original) The method of claim 143, wherein said method further comprises the step of amplifying said extended product by polymerase chain reaction.

145. (Original) The method of claim 140, wherein said population of DNA molecules is obtained from serum.

146. (Original) The method of claim 140, wherein said population of DNA molecules is obtained from plasma.

147.-152. (Cancel)